Rapid Extraction of Multiple RNA Samples from Plant Suspension Cells

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Extraction of nucleic acids from plant cells is a labor-intensive and time-consuming process. The main reason is the cell wall that must be broken, typically by mechanical means, so that extraction buffers can exert their effect. Grinding, mechanical homogenization or repeated pipetting procedures are cumbersome, especially when handling multiple samples, and inherently cause variation in yield and quality of RNA obtained.

We have extended the single-step RNA isolation method based on guanidine isothiocyanate/phenol/chloroform, described by Simms *et al.* (1) for animal cells and tissues, to plant cells. We have found that TRIZOL™ Reagent can be used to extract total RNA from alfalfa suspension cells without the need for grinding or any other form of mechanical breaking of the plant cell wall.

Experimental Procedures

Alfalfa (*Medicago varia*) A2 suspension cells (2) in early to late log phase were collected into a 0.5- to 1.0-ml pellet from 5 to 15 ml culture by centrifugation at 150 × *g* for 4 min at 20°C in a capped, sterile, 15-ml polypropylene tube. The culture medium was decanted and the tube with cell pellet was placed directly in a –70°C freezer until use. RNA extraction was performed in lots of eight tubes. GIBCO BRL TRIZOL Reagent (2.5 ml) was pipetted on top of the frozen pellet and the tubes were placed in a waterbath of 37°C for 3 min with vigorous shaking by hand every min for 10 s. Subsequently, the eight tubes were vortexed together for 2 min at room temperature. After 5 min at room temperature, 0.5 ml chloroform was added to each tube and shaken vigorously by hand for 10 s. Room temperature incubation was continued for 3 min. The tubes were centrifuged at 4,000 × *g* for 25 min at 4°C. At this time, RNA isolation from the next batch of eight samples was started. The colorless upper phase in each tube was collected and RNA was precipitated with 1.25 ml isopropanol and washed with 4 ml of 75% ethanol according to Simms *et al.* (1). RNA was solubilized in 80 µl DEPC-treated 0.5% (w/v) SDS in water.

Total RNA was electrophoresed in agarose gels under nondenaturing conditions and under denaturing conditions in formaldehyde (3). Northern analysis of alfalfa histone H3 transcripts was performed with gene-specific, digoxigenin-labeled hybridization probes, which were visualized by chemiluminescence.

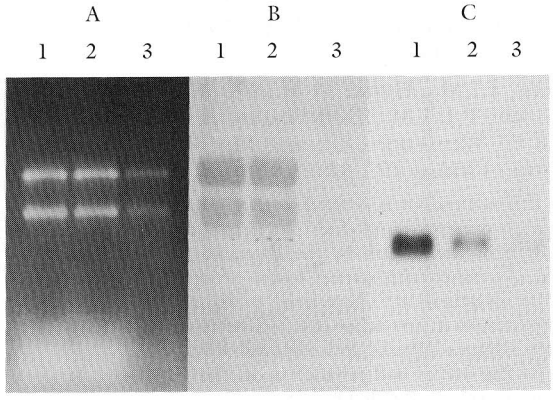

FIGURE 1. Analysis of RNA recovered from alfalfa suspension cells or callus. Panel A. Total RNA (2 µg), obtained from alfalfa A2 cells at two different times from a partially cell-cycle-synchronized suspension culture (lanes 1 and 2), was electrophoresed in Tris-borate-EDTA buffer and stained with ethidium bromide. Lane 3. Total RNA obtained from an equivalent amount of alfalfa callus, cultured on plates. For Northern analysis, 7.5 times more RNA was separated on an agarose-formaldehyde gel. Panel B. Transferred RNA stained with methylene blue (4). Panel C. The membrane was hybridized with a digoxigenin-substituted alfalfa histone H3 probe that is specific for histone H3.1 (2).

Results and Discussion

Plant cell suspension culture conditions produce small clusters of vacuolized cells, surrounded by cell walls. Freezing followed by rapid thawing at 37°C in excess TRIZOL Reagent, a strong chaotropic solvent with guanidine isothiocyanate, produced effective cell lysis with simple mixing. Mechanical breakage of cell walls was not required. Thus, it is possible to prepare intact total RNA from many small samples, in reproducibly high yields (fig-

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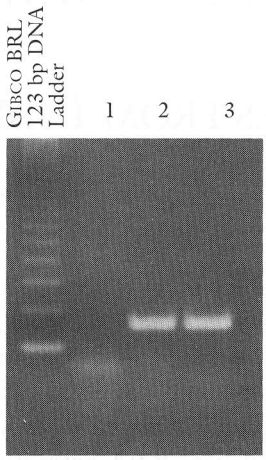

FIGURE 1. RT-PCR of extracted RNA from equine semen samples. Ethidium-bromide-stained agarose gel. Lane 1, EAV-negative equine semen. Lanes 2 and 3, EAV-positive equine semen.

as 2.5 pfu of EAV/ml semen gave equally intense product bands (data not shown). No product bands were observed from the negative semen (lane 1). Furthermore, the number and size of restriction fragments obtained after enzymatic digestion of the RT-PCR product obtained from positive semen samples were consistent with those predicted for virus-specific cDNA amplification (data not shown).

Based on the findings of an initial study, in which tissue culture-derived EAV was used to spike semen, RT-PCR could detect as little as 0.01 pfu/ml, as demonstrated by ethidium bromide staining of the product. This suggests that viral RNA not associated with infectious particles was also being detected. Thus, the results indicate that the TRIzol Reagent extraction for viral RNA was efficient and that the procedure could be used in an RT-PCR assay for the detection of viral RNA in equine semen samples.

Methods for RT-PCR for the detection of EAV-specific RNA in semen have been reported previously (1,7). In each case, the RNA extraction procedure was more involved and time consuming than that described herein, and it included overnight or double precipitations of the RNA. Previously, cellular RNA isolated with TRIzol Reagent was used successfully for RT-PCR (3). In this report, we extend the use of the reagent to the isolation of viral RNA from semen. This involved the simple addition of TRIzol Reagent directly to seminal plasma containing glycogen.

In addition to the isolation of viral RNA from seminal plasma, we have also used this procedure successfully for the extraction of RNA from EAV and a related RNA virus, porcine reproductive and respiratory syndrome virus (8), directly from infected tissue culture supernatants for RT-PCR assays. It is anticipated that this procedure will be generally useful in the extraction of viral RNA from a variety of biological sources for RT-PCR.

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ure 1). Additional homogenization of TRIZOL Reagent lysates failed to increase the yield of RNA (data not shown). The volume of TRIZOL Reagent that was required for plant cell lysis, a 2.5-fold excess, is less than the 10-fold excess recommended for the preparation of RNA from animal cells (1). The minimal amount of reagent required was established experimentally for our plant cell culture and may vary with factors such as the buffering capacity of cells and growth medium. In general, 0.5 to 1.0 mg total RNA ($A_{260/280}$ = 2.0-2.1) was obtained from 1 ml cell pellet, corresponding to 50 mg lyophilized tissue. This yield of RNA from plant is similar to that obtained with more conventional, laborious methods (2).

When callus cultures, grown on agar plates, were used, considerably less RNA was obtained (figure 1, lane 3). The yield of RNA was increased if a mechanical homogenization step was added (data not shown). This suggests that the large cluster of cells in the callus impedes access of the chaotropic buffer to the deeper cell layers.

RNA isolated with TRIZOL Reagent was not degraded, as judged by the pattern of ribosomal RNAs in the gel when stained with ethidium bromide, and after transfer of the RNA to a membrane and stained with methylene blue. Also, hybridization with an alfalfa histone H3 gene probe produced a sharp band (figure 1, panel C).

In summary, TRIZOL Reagent is fast, inexpensive, and produces high-quality RNA from multiple samples in reproducibly high yields.

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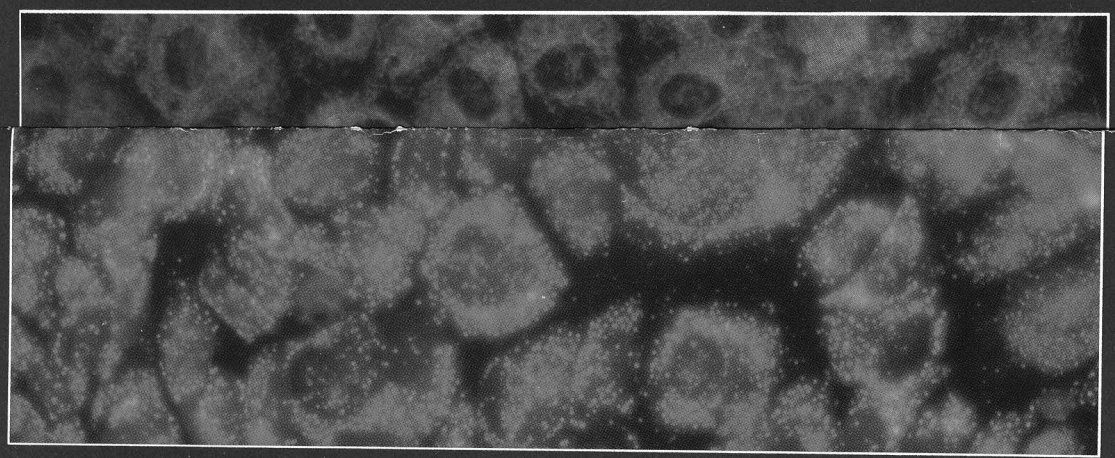

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